Attorney Docket No.: 5835.200-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Bisgård-Frantzen et al Confirmation No: 9183

Serial No.: 09/710,339 Group Art Unit: 1651

Filed: November 9, 2000 Examiner: Monshipouri, M.

For: Fungamyl-like Alpha-Amylase Variants

CERTIFICATE OF MAILING UNDER 37 CFR 1.8(a)

United States Patent and Trademark Office
Room 10A 17
Crystal Mall
1911 South Clark Place
Arlington, VA 22202
Attn: Examiner M. Monshipouri

O.K. to enter R.N.

Sir:

I hereby certify that the attached correspondence comprising:

1. Declaration under 37 C.F.R. 1.132
2. Figure

is being hand delivered addressed to the address indicated above on November 21, 2003.

Julie Tabarovsky
(name of person mailing paper)

Julie Tabarovsky
(signature of person mailing paper)

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The Examiner requested that Applicants provide some additional clarifying information to address the obviousness rejection set forth in the Office Action mailed March 21, 2003. Enclosed is a declaration by co-inventor Allan Svendsen, providing the requested information.

If there are any questions, the Examiner is encouraged to contact the undersigned.

Respectfully submitted,

Date: November 19, 2003

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(212)840-0097

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DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Allan Svendsen, do hereby state and declare that

1. I am a citizen of Denmark residing in Horsholm, Denmark. I have a degree in Biochemistry from the Institute of Biochemical Genetics, University of Copenhagen, Denmark. I currently hold the position of Senior Science Manager of Protein Design at Novozymes A/S, Bagsvaerd, Denmark. I am an author or co-author of more than 80 scientific publications and I am an inventor or co-inventor on more than 60 patent applications and patents in the field of protein engineering, including alpha-amylase engineering. I am also the editor of the recent textbook entitled Enzyme Functionality: Design, Engineering, and Screening

2. I am a co-inventor of the above-captioned application, directed to fungal-related alpha-amylase variants. I am also a co-inventor of the subject matter disclosed in Svendsen et al., WO 96/23874, directed to bacterial-related alpha-amylase variants, which is now relied upon by the United States Patent Office for the obviousness rejection.

3. The alterations in the fungal related alpha-amylases claimed in the above-captioned patent application cannot be predicted from the bacterial related alpha-amylase variants described in WO 96/23874. Based on an amino acid sequence comparison, the fungal-related alpha-amylases involved in the present invention share only very low amino acid sequence homology to the bacterial-related alpha-amylases of WO 96/23874, generally less than about 50%. Because of the low sequence similarity, alterations in bacterial alpha-amylases

usually cannot reasonably predict alterations in fungal alpha-amylases. Even though the enzymes share a common protein fold, the very low homology results in very different amino acid interactions, including the large difference in three-dimensional structure, as discussed below.

4. The difficulty of predicting suitable alternations based on proteins which have very low amino acid sequence homology is further evidenced and clearly illustrated by a three dimensional comparison between representative proteins of the present invention and WO 96/23874. Accompanying my declaration is a structural comparison of the bacterial alpha-amylase, *Bacillus licheniformis* alpha-amylase, representative of the alpha-amylases of WO 96/23874, and the fungal alpha-amylase, *Aspergillus oryzae* alpha-amylsae, representative of the alpha-amylases of the claimed invention. The comparison provides two different perspectives (Perspective A and Perspective B) of the fungal alpha-amylase three dimensional structure superimposed on the bacterial alpha-amylase three dimensional structure (the fungal alpha-amylase is shown in dark blue and the bacterial alpha-amylase is shown in light blue). The structures were structurally aligned by superimposing the 8 beta strands of the central beta-barrel using the superimpose program in INSIGHTII (Accelrys, Inc.) Although there are a few structural similarities between bacterial and fungal alpha-amylases, including, the central beta-barrel and the active site residues, and to a lower degree a few peptide stretches, especially in the A domain, there are many more significant structural differences between fungal and bacterial alpha-amylases, such that the overall structures are considered to be very different. Some of these significant structural differences include nearly all structural parts outside the central beta-barrel and outside the active site residues, especially in the very different folding of the B domain and in the ion binding sites (1 calcium ion in the fungal alpha-amylase and 3 calcium ions in the bacterial alpha-amylase +_1 sodium ion). The differences are seen clearly in the very different interactions found in the spatial arrangements of the two alpha-amylases.

5. In addition to the fact that the fungal and bacterial alpha-amylases have a very different overall three dimensional structure, the attached three-dimensional comparison also illustrates the significant structural differences at the specific regions recited in our claims, namely, regions 98-110, 150-160, 161-167, 280-288, 448-455 and 468-475. In particular, as can be seen from the attached three-dimensional comparison, regions 150-160, 161-167, 280-288, 448-455 and 468-475 are very different in both their structure and location from the possible corresponding regions in the bacterial alpha-amylase. Indeed, many of these regions do not have substantially corresponding regions in the referenced bacterial alpha-amylase, as is

apparent from the lack of overlap between the structures. Thus, it is not reasonable to extrapolate reliable conclusions about whether changes in the bacterial alpha-amylase amino acids would predict suitable corresponding changes in a fungal alpha amylase at these regions.

Region 98-110 is some what less different from the corresponding region in the bacterial alpha-amylase as there is also a helix formed in the bacterial alpha-amylase in the A domain. Nevertheless, as can be seen from the comparison, there are still significant differences between the structure and location of region 98-110 and the possible corresponding region in the bacterial alpha-amylase such that the skilled artisan would not be able to predict with a reasonable expectation of success the suitability of the claimed alterations in fungal alpha-amylases based on WO 96/23874. The differences between region 98-110 and the structure of the bacterial alpha-amylase is shown more clearly in Perspective B, which illustrates that although both structures have helices in domain A, in the context of the entire molecule, some of the helices are actually located spatially in very different areas within the molecules, including the region 98-110, resulting in a difference in residue-residue interactions to the helix.

6. Thus, it is my conclusion that the alterations claimed in the above-captioned application directed to fungal-related alpha amylases cannot be predicted from the bacterial-related alpha-amylase variants of WO 96/23874, as evidenced by, among other things, the significant overall structural differences as well as the specific structural differences at the regions claimed in the above-captioned patent application.

7. The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize any patent issuing thereon.

Signed this 19 day of November, 2003

Allan Svendsen